Mycobiology

Research Note

First Report of Potato Stem-End Rot Caused by *Fusarium oxysporum* in Korea

Md. Aktaruzzaman[1], Sheng-Jun Xu[1], Joon-Young Kim[1], Jae-Hyoun Woo[1], Young-Il Hahm[2] and Byung-Sup Kim[1,*]

[1]*Department of Plant Science, Gangneung-Wonju National University, Gangneung 210-702, Korea*
[2]*Daegwallyong Horticultural Cooperative, Pyungchang 232-950, Korea*

Abstract In this study, we identified the causative agent of stem-end rot in potatoes that were grown in Gangwon alpine areas of Korea in 2013. The disease symptoms included appearance of slightly sunken circular lesion with corky rot on the potato surface at the stem-end portion. The fungal species isolated from the infected potatoes were grown on potato dextrose agar and produced white aerial mycelia with dark violet pigments. The conidiophores were branched and monophialidic. The microconidia had ellipsoidal to cylindrical shapes and ranged from 2.6~11.4 × 1.9~3.5 μm in size. The macroconidia ranged from 12.7~24.7 × 2.7~3.6 μm in size and had slightly curved or fusiform shape with 2 to 5 septate. Chlamydospores ranged from 6.1~8.1 × 5.7~8.3 μm in size and were present singly or in pairs. The causal agent of potato stem-end rot was identified as *Fusarium oxysporum* by morphological characterization and by sequencing the internal transcribed spacer (ITS1 and ITS4) regions of rRNA. Artificial inoculation of the pathogen resulted in development of disease symptoms and the re-isolated pathogen showed characteristics of *F. oxysporum*. To the best of our knowledge, this is the first study to report that potato stem-end rot is caused by *F. oxysporum* in Korea.

Keywords *Fusarium oxysporum*, Pathogenicity, *Solanum tubersum*, Stem-end rot of potato

Potato (*Solanum tubersum* L.) is one of the most significant and globally grown crops and has an important role in human nutrition. The potato ranks as the world's fourth largest food crops after wheat, rice and corn [1]. In 2011, potato was grown in Korea on 26,804 ha of land to yield a total of 622,230 Mt of potato, with productivity of 23.21 t/ha [2]. Potatoes are consumed as a staple food or are processed into food products, food ingredients, or industrial starch. Potatoes are also re-used as seed tubers for growing potato crop for the following season.

Fusarium is a large genus that belongs to the Ascomycota phylum and comprises a few hundred species that are mainly distributed in soils or are associated with plants [3]. The *Fusarium* genus consists of a species complex that has several clonal lineages [4]. It has a global distribution and is responsible for severe vascular wilts, and can cause rots of various plant structures such as roots, stalks, cobs, seedlings, tubers, bulbs, and corms of a wide range of plants [4, 5]. In addition, *Fusarium* species cause post-harvest dry rot and stem-end rot during the growing season in potato [6-9]. *Fusarium oxysporum* growth is favored by dry soil conditions and the optimum soil temperature for infection is 15°C [10]. In September 2013, potatoes harvested from commercial fields of Gangwon alpine areas presented symptoms of potato stem-end rot. In this study, we identified the causative agent of potato stem-end rot and characterized the pathogenicity of the isolated pathogen.

Mycobiology 2014 June, **42**(2): 206-209
http://dx.doi.org/10.5941/MYCO.2014.42.2.206
pISSN 1229-8093 • eISSN 2092-9323
© The Korean Society of Mycology

*__Corresponding author__
E-mail: bskim@gwnu.ac.kr

Received February 15, 2014
Revised May 22, 2014
Accepted May 29, 2014

Isolation of the fungi and pathogenicity test. Stem-end rot disease-infected potato tubers were collected from commercial potato growing areas of Gangwon-do (Korea) in September 2013. The disease-infected tubers with corky rot had slightly sunken circular lesions with sharply defined margins and had white fungal mycelium growth on the surface of the stem-end portions of the potatoes (Fig. 1A). Infected tubers were collected in sterilized plastic polythene bags and transported to the laboratory for pathogen isolation. Infected tubers were cut into small pieces of approximately 1.0~1.5 cm size. The potato pieces were surface-sterilized with 0.1% sodium hypochlorite (NaOCl) for one minute, washed three times with sterile distilled water, and then dried with sterilized filter paper. Next, the potato pieces

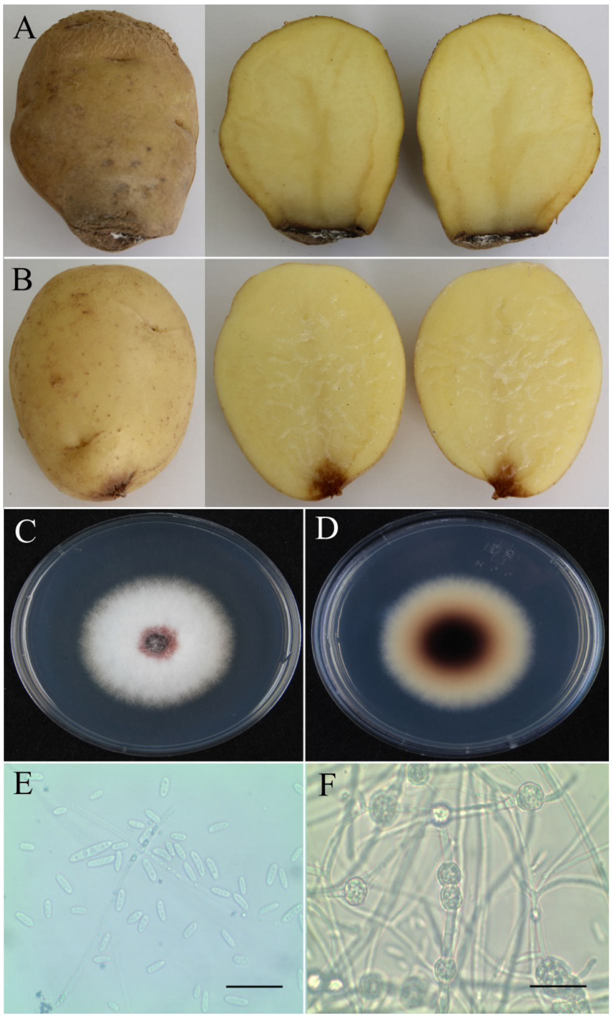

Fig. 1. A, Disease-infected potato showing a slightly sunken circular lesion with corky rot; B, Potato inoculated with *Fusarium oxysporum* developed stem-end rot symptoms after 7 wk of incubation; C, D, One-week-old colony of *F. oxysporum* growing on potato dextrose agar medium; E, Macroconidia and microconidia; F, Chlamydospores (scale bars: E, F = 20 μm).

were placed in Petri plates containing potato dextrose agar (PDA) medium (Difco, Detroit, MI, USA) and incubated at 25 ± 2°C for 5 days. For pure culture isolation, the mycelia growth obtained on the PDA plates was used to inoculate fresh PDA plates.

To characterize the pathogenicity of the fungus, 40-day-old potato plants cv. Superior were transplanted into plastic pots containing commercial soil (Baroker; Seoul Bio Co., Ltd., Eumseong, Korea). Two weeks after planting the potato plants, 25 mL *F. oxysporum* conidial suspension (adjusted to 1×10^6 conidia per mL by using a hemocytometer and prepared by suspending conidia from PDA cultures in sterile water) was applied by soil drenching. The greenhouse temperature was maintained at 25 ± 2°C and the plants were watered twice weekly. Seven weeks after inoculation, small, purple-brown or light yellow corky rot developed on the potato tubers at the stem-end portions (Fig. 1B). The fungal pathogen was re-isolated from the disease lesions of the inoculated plants and the re-isolated pathogen exhibited the same morphological characteristics as those of the original isolates. Thus, the fungal pathogen fulfilled the four criteria stipulated by the Koch's postulates and was identified as the causative agent of the potato stem-end rot.

DNA extraction, PCR, and sequence analysis. The pathogen was isolated from the disease lesion of the potatoes by using the surface sterilization method. Mycelia were obtained from a 7-day-old culture on PDA. For DNA extraction, mycelia were grown in 250-mL flasks containing 100 mL potato dextrose broth, which were incubated for 6 days at 25°C on a rotary shaker at 150 rpm. Mycelia were harvested by vacuum filtration through Whatman grade 1 filter paper and then lyophilized for 24 hr before grinding them to a fine powder. Next, 100 mg of the ground powder was transferred to a 1.5-mL Eppendorf tube and DNA was extracted by using the CTAB extraction method [11].

The extracted DNA was used for PCR sequencing of rDNA genes by using universal primers for internal transcribed spacer (ITS) 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) [12]. The amplification was performed in a 25 μL reaction mixture containing 0.5 μL of each primer, 0.5 μL of *Taq* DNA polymerase (Bioneer, Daejeon, Korea), 0.5 μL of each dNTP, 2.5 μL of 10× PCR reaction buffer, 18.5 μL of distilled water, and 2.0 μL of template DNA. The reaction was performed in Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) under the following conditions: pre-denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 35 sec, annealing at 52°C for 55 sec, and elongation at 72°C for 1 min and then final extension at 72°C for 10 min [13]. The obtained nucleotide sequences were searched through BLASTN at the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic analysis of *F. oxysporum* was performed by using the MEGA5 program with the neighbor-joining method [14]. Sequence data were deposited in GenBank (accession No. KJ162149).

Identification and characterization of *F. oxysporum*. Total 5 fungal strains were obtained from the stem-end root of potato, and Strain F2 among 5 strains was examined for identification. Strain F2 was identified as *F. oxysporum* by analyzing the morphological characteristics of the isolated fungus and by performing rDNA sequencing analysis. The fungal colonies were isolated on PDA and consisted of white aerial mycelia that later produced dark violet pigments that are characteristic of *F. oxysporum* (Fig. 1C and 1D). *F. oxysporum* has three types of conidia: macroconidia, microconidia, and chlamydospores. On the other hand, other *Fusarium* spp. have only two types of conidia: macroconidia and chlamydospora. Shapes of macroconidia and microconidia (Fig. 1E), conidiophores and chlamydospores (Fig. 1F) are

Table 1. Comparison of morphological characteristics of the study isolate with respect to previously reported *Fusarium oxysporum* characteristics

Characteristics		Study isolate	*F. oxysporum*[a]
Conidiophores	Shape	Branched monophialides	Unbranched and branched monophialides
Microconidia	Shape	Ellipsoidal to cylindrical straight or slightly curved	Oval-ellipsoidal, straight to curved
	Septa	No septate	No septate
	Length (μm)	2.6~11.4	5.0~12
	Width (μm)	1.9~3.5	2.2~3.5
Macroconidia	Shape	Fusiform, more or less curved	Fusoid-subulate
	Septa	2~5 pointed at both ends	Usually 3~5 septate pointed at both ends
	Length (μm)	12.7~24.7	27~46
	Width (μm)	2.7~3.6	3.0~4.5
Chlamydospores	Shape	Globose-ellipsoidal, singly or in pairs	Globose-ellipsoidal, singly or in pairs
	Length (μm)	6.1~8.1	4.0~16
	Width (μm)	5.7~8.3	4.0~12

[a]Described by Booth [15].

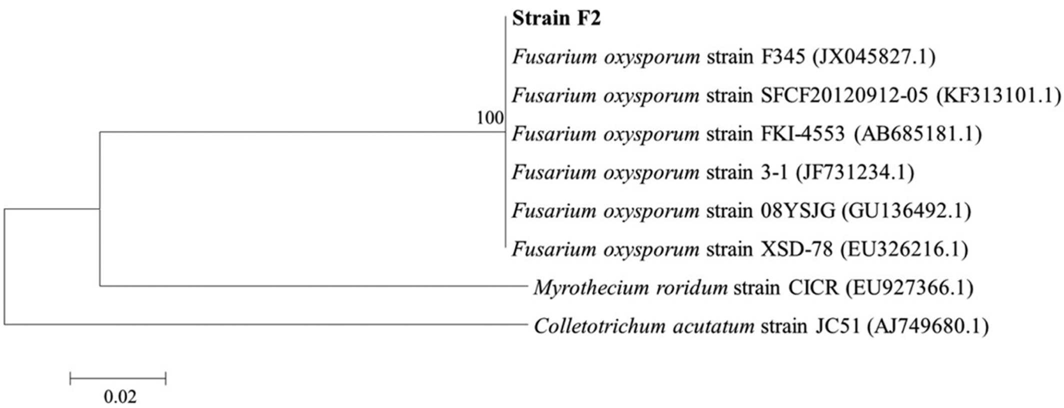

Fig. 2. Neighbor-joining phylogenetic tree of *Fusarium oxysporum* and related species identified from GenBank based on internal transcribed spacer gene sequences numbers at the nodes indicate bootstrap values from a test of 1,000 replications. The scale bar indicates the number of nucleotide substitutions. Evolutionary analyses were conducted by using the MEGA5 program [14].

distinctive characteristics of *F. oxysporum*. The morphological characteristics of the identified species are summarized in Table 1. The ITS sequence was compared to the GenBank database sequences by using the NCBI BLAST search tool. The sequences identified based on rRNA-ITS alignment were 100% similar to those of several *F. oxysporum* species (accession Nos. JX045827.1, KF313101.1, AB685181.1, JF731213.1, GU136492.1, and EU326216.1). Thus, *F. oxysporum* was identified as the causative agent of potato stem-end rot in Korea (Fig. 2). This is the first report of stem-end rot of potato tubers in Korea.

REFERENCES

1. Muthoni J, Nyamongo DO. A review of constraints to ware Irish potatoes production in Kenya. J Hortic For 2009;1:98-102.
2. Information and Statistics Division of Korean Ministry for Food, Agriculture, Forestry and Fisheries. Food, agriculture, forestry and fisheries statistical year book 2012. Gwacheon: Korean Ministry for Food, Agriculture, Forestry and Fisheries; 2012. p. 100-1.
3. Summerell BA, Laurence MH, Liew EC, Leslie JF. Biogeography and phylogeography of *Fusarium*: a review. Fungal Divers 2010;44:3-13.
4. Michielse CB, Rep M. Pathogen profile update: *Fusarium oxysporum*. Mol Plant Pathol 2009;10:311-24.
5. Enya J, Togawa M, Takeuchi T, Yoshida S, Tsushima S, Arie T, Sakai T. Biological and phylogenetic characterization of *Fusarium oxysporum* complex, which causes yellows on *Brassica* spp., and proposal of *F. oxysporum* f. sp. *rapae*, a novel forma specialis pathogenic on *B. rapa* in Japan. Phytopathology 2008;98:475-83.
6. Garcia Bayona L, Grajales A, Cardenas ME, Sierra R, Cepero de Garcia MC, Bernal A, Jimenez P, Restrepo S. First report of *Fusarium oxysporum* causing potato dry rot in *Solanum tuberosum* in Colombia. New Dis Rep 2011;24:14.
7. Theron DJ, Holz G. *Fusarium* species associated with dry and stem-end rot of potatoes in South Africa. Phytophylactica 1989;21:175-81.
8. Brewer PJ. Potato stem-end rot caused by *Fusarium* spp. S

Afr J Agric Sci 1962;5:475-81.
9. Pont W. Studies on tuber stem end rot and stem rot of potato in north Queensland caused by *Fusarium solani* and *Fusarium oxysporum*. Queensland J Agric Anim Sci 1976;33:129-44.
10. MacMillan HG, Meckstroth GA. The critical temperature for infection of the potato seed piece by *Fusarium oxysporum.* J Agric Res 1925;32:917-21.
11. Möller EM, Bahnweg G, Sandermann H, Geiger HH. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Res 1992;20:6115-6.
12. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.
13. Nishizawa T, Zhaorigetu, Komatsuzaki M, Sato Y, Kaneko N, Ohta H. Molecular characterization of fungal communities in non-tilled, cover-cropped upland rice field soils. Microb Environ 2010;25:204-10.
14. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731-9.
15. Booth C. *Fusarium oxysporum.* CMI descriptions of pathogenic fungi and bacteria. Kew, Surrey: Commonwealth Mycological Institute; 1970.